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September 30, 1982

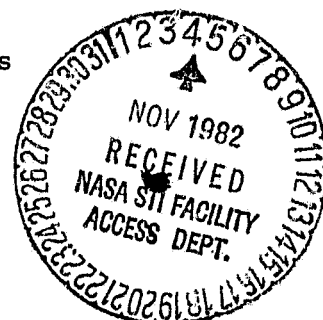
ANNUAL PROGRESS REPORT

Principal Investigator: D.E. Brooks, Ph.D.

Project Title: Countercurrent Distribution of Biological Cells

Reporting Period: August 1, 1981-July 31, 1982

Contract No.: NAS8-33575



Progress During Reporting Period

A. Summary

1. Phosphate partitioning measurements were conducted on a series of dextran T40-PEG 6000 aqueous two-phase polymer systems up to 0.22 M total phosphate concentration. It was observed that phosphate partition coefficient increases at higher phosphate concentrations for systems of the same tie line length.

2. In order to investigate the type of phosphate-polymer interaction which produces the phosphate partitioning the individual polymers were dialyzed against various concentrations of phosphate. It was found that while dextran did not influence the phosphate concentration in the dialysis bag, PEG on the other hand exerted a marked rejection of the salt which was an increasing function of PEG concentration. For the detailed information please see the two attached manuscripts entitled, respectively:

- a. S. Bamberger, G.V.F. Seaman, J.A. Brown and D.E. Brooks. The partition of sodium phosphate and sodium chloride in aqueous dextran poly(ethylene glycol) two phase systems. Submitted to the Journal of Colloid and Interface Science.
- b. D.E. Brooks and S. Bamberger. Studies on aqueous two polymer systems useful for partitioning of biological materials. In: Materials Processing in the Reduced Gravity Environment of Space (Guy E. Rindone, ed.), Elsevier Publishing Co., New York, 1982, pp. 233-240.

3. The effectiveness of the separation in the field-driven phase separation was determined directly by separating the phase system at the position of the interface and measuring the amount of upper and lower phase in the top and bottom compartment at various time periods after mixing. As demonstrated in Figure 1 the electrical field only slightly accelerates the phase segregation in the initial very fast separation period, which occurs during the first 0.5 minutes. However for longer settling times, the electrical field-driven phase separation is much more effective.

B. Problems Encountered During the Reporting Period

Some of the objectives which have to be met for this project are unlikely to be completed on schedule and a request is being prepared therefore for a no cost extension to December 31, 1982.

(NASA-CR-170652) COUNTERCURRENT
DISTRIBUTION OF BIOLOGICAL CELLS Annual
Progress Report, 1 Aug. 1981 - 31 Jul. 1982
(British Columbia Univ.) 28 p HC A03/MF A01

N83-11330

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CSCL 11G G6/27 01027

C. Activities Planned for the Next Reporting Period

1. To study whether sulfate interacts with PEG and dextran in a similar manner to phosphate.

2. To examine partitioning of erythrocytes in the field-driven phase separation apparatus with and without application of the electrical field.

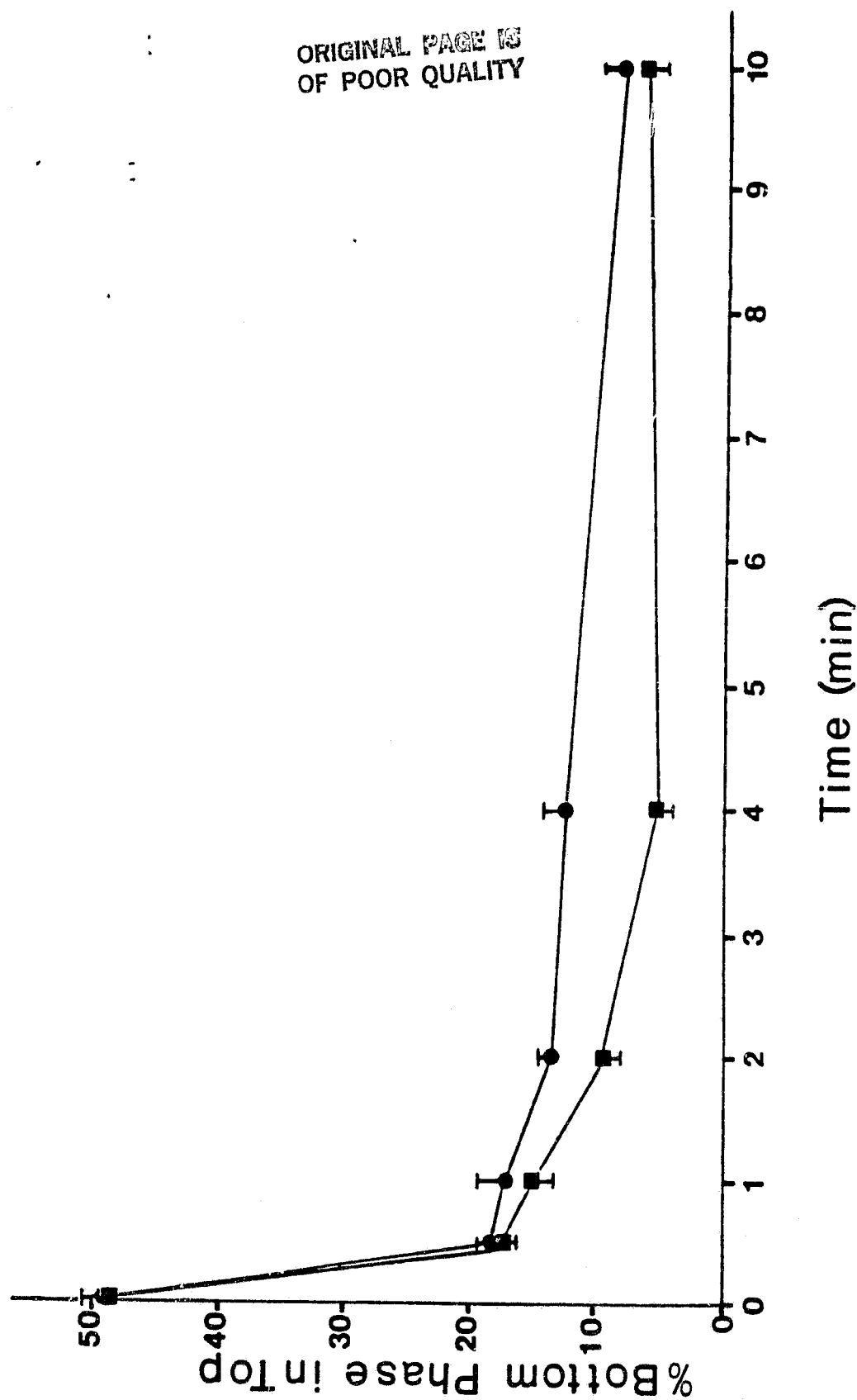


Figure 1: Time course of phase separation in the sample chamber in the presence (■) and absence (●) of an applied electric field of 0.5 V cm^{-1} . The phase system used contained 7.5% dextran T40, 4.5% PEG 6000 and 0.11 M sodium phosphate pH 7.5.

THE PARTITION OF SODIUM PHOSPHATE AND SODIUM CHLORIDE
IN AQUEOUS DEXTRAN POLY(ETHYLENE GLYCOL) TWO PHASE SYSTEMS

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Running Title: Salt Effects on Two Phase Polymer Systems

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ABSTRACT

It is known that the addition of phosphate buffer to two polymer aqueous phase systems has a strong effect on the partition behavior of cells and other particles in such mixtures. The addition of sodium phosphate to aqueous poly(ethylene glycol) dextran phase systems causes a concentration-dependent shift in the binodial on the phase diagram, progressively lowering the critical conditions for phase separation as the phosphate concentration is increased. Sodium chloride produces no significant shift in the critical point relative to the salt-free case. Accurate determinations of the phase diagram require measurements of the density of the phases; data is presented which allows this parameter to be calculated from polarimetric measurements of the dextran concentrations of both phases. Increasing polymer concentrations in the phase systems produce increasing preference of the phosphate for the dextran-rich bottom phase. Equilibrium dialysis experiments showed that poly(ethylene glycol) effectively rejected phosphate, and to a lesser extent chloride, but that dextran had little effect on the distribution of either salt. Increasing ionic strength via addition of 0.15 M NaCl to phase systems containing 0.01 M phosphate produces an increased concentration of phosphate ions in the bottom dextran-rich phase, the expected effect in this type of Donnan distribution.

INTRODUCTION

Polymer two phase aqueous systems have been used successfully for the separation of cells, cell organelles and macromolecules (1). Because the partitioning of such material has proved to be strongly dependent on the type and concentration of salt added to the system, the distribution of various salts themselves has been studied (2) in systems containing dextran and PEG. It was found that polyvalent anions such as phosphate, sulfate and citrate partitioned preferentially into the dextran-rich bottom phase while halides partitioned nearly equally (2). As a result of the salt concentration gradient thus set up, the addition of sulfate or phosphate to dextran/PEG phase systems induces an electrostatic potential difference between the two phases, the magnitude of which depends on the salt concentration (3,4). Furthermore, electrophoretic measurements of drops of one phase suspended in the other phase show that the electrophoretic mobility of the drops increases, for example, with increasing phosphate concentration (5). Since cell partitioning experiments in dextran/PEG phase systems often involve the use of phosphate to buffer the systems and to make them isotonic, we have carried out a more detailed examination of the effects caused by this salt.

MATERIALS AND METHODS

The polymer stock solutions contained 25-30% w/w of either Dextran T40 ($\bar{M}_w = 41,500$, $\bar{M}_n = 26,800$; Pharmacia Fine Chemicals, Uppsala, Sweden; lot 7974), Dextran T500 ($\bar{M}_w = 511,000$, $\bar{M}_n = 191,600$; Pharmacia; lot 3447), PEG 6 ($\bar{M}_w = 6,900$; Union Carbide, New York, NY; lot R-529-9104) or PEG 20 ($\bar{M}_w = 20,000$; Union Carbide; lot 900 1S568182). The phosphate stock solutions were made by mixing aqueous solutions of sodium dihydrogen phosphate and disodium hydrogen phosphate of equal concentrations until the desired pH was reached.

The phase systems were made by mixing appropriate weights of polymer stock solutions with stock salt solutions and making up to the desired w/w concentration with twice distilled water in a graduated cylinder. The phase systems were allowed to separate at the desired temperature until neither phase showed visible turbidity. Following removal of all but 3 to 5 ml of top phase, the remaining top phase together with a few milliliters of the bottom phase was aspirated and discarded. Then the lower phase was drawn off into a syringe with a long needle from the bottom of the graduated cylinder, leaving behind the last 3 to 5 ml. The buoyant densities of the phases were obtained by pycnometry with an estimated error of ± 0.2 mg/ml.

The dextran concentrations in the phases and in the stock solutions were determined by polarimetry (Circle Polarimeter 0.05° , Carl Zeiss, Oberkochen, West Germany) with a precision of $\pm 0.02\%$ w/v. The PEG concentrations in the stock solutions were calculated from the refractive indices of the solutions to a precision of $\pm 0.1\%$ w/v. Since the refractive index increases linearly with the w/v concentrations of the polymers, as well as of the salt, the PEG concentrations in the phases were obtained by subtracting the respective increments for dextran and for the salt from the measured values. Dextran specific volume was determined pycnometrically.

Phosphate concentrations were determined by the stannous chloride method (8) and were reproducible to within $\pm 3\%$. Because PEG precipitates and interferes with the assay, the organic material in the phases had to be removed by digestion in a mixture of concentrated nitric and sulfuric acids according to the procedure described by Taras (6). Occasionally the digestion did not completely oxidize the organic matter. In these cases additional concentrated nitric acid was added to the cooled reaction mixture and the

digestion was repeated. The chloride concentrations were determined with an Aminco-Cotlove automatic chloride titrator (American Instrument Co., Silver Springs, MD) and were reproducible to within $\pm 1\%$.

For the equilibrium dialysis experiments, dialysis tubing (Spectra/Por 6, molecular weight cutoff 2000, 64 mm diameter; Spectrum Medical Industries, Inc., Los Angeles, CA) was enclosed in a pouch of nylon netting to provide mechanical support. The tubing was filled with 1 to 2 ml of 40 to 50% w/w polymer stock solutions. The dialysis was carried out for 24 to 36 hours against 1 l of 0.11 M or 0.22 M sodium phosphate, pH 7.5, or 0.15 M NaCl, with the salt solutions changed once. Neither the addition of phosphate to the polymer stock solutions nor the increase of the dialysis period to 48 hours had significant effect on the results.

All measurements were carried out at room temperature in an air conditioned laboratory, $22 \pm 1^\circ\text{C}$.

RESULTS

The optical methods used to determine the polymer concentrations provide w/v concentrations. To derive the % w/w values the densities of the phases have to be known. Figures 1 and 2 show that both the dextran concentration versus density of the bottom, dextran-rich phase and the difference in dextran concentration versus the difference in density between the two phases follow linear relationships. Similar linear relationships are found if the PEG concentration of the top phase is plotted versus the bottom phase density or the difference in PEG concentration is plotted versus the density difference between the phases, but not if the bottom phase dextran or the top phase PEG concentration is plotted versus the top phase density. In the top phase the density changes only slightly with changing polymer concentrations. For example, in systems

containing no salt the highest density of 1.026 g/ml was found in the system closest to the critical point. With increasing total polymer concentration the top phase density first decreases, reaching a minimum of 1.021 g/ml in 12.2% w/w Dextran T40 and 3.5% w/w PEG 6, then increases slightly to 1.022 g/ml at the highest polymer concentration investigated (14% w/w Dextran T40, 5% w/w PEG 6). The linear relationships given in Figs. 1 and 2 can be used to calculate the densities of all phases in the systems studied.

Figure 3 shows the binodials of the phase diagrams for Dextran T40/PEG 6 systems at different salt concentrations. The binodials for the systems with 0.15 M NaCl and without any salt added are so close to each other that only one line was drawn.

One parameter which can be used to describe the degree of phase separation is the length of the tie line which joins the two points on the binodial which describe the composition of the two phases. It is seen from Fig. 4 that as the polymer system approaches the critical point the tie line length is increasingly influenced by added salt.

Figure 5 shows that with increasing tie line length, phosphate partitions increasingly into the bottom, dextran-rich phase. The linear regression analyses for systems containing 0.11 M and for 0.22 M phosphate yield essentially parallel lines. That the lines are distinct indicates that the phosphate concentration, or ionic strength, influences the partitioning, the distribution becoming more one-sided the greater the amount of phosphate in the system. This is confirmed by the phosphate distribution in systems with 10 mM phosphate, pH 7.5, the values of which are all above those of systems with higher phosphate concentrations but having the same tie line length. However, if the ionic strength is increased with an indifferent salt, in this case 0.15 M NaCl in addition to 0.01 M phosphate, the top/bottom ratio drops slightly below that

for 0.22 M phosphate.

In some systems containing 0.15 M NaCl and 0.01 M sodium phosphate, pH 7.5 (5, 6, 6.5 or 7% w/w Dextran T500 and 4% PEG 6), the chloride concentrations in the top and bottom phase were determined. In all cases the top/bottom concentration ratios fluctuated around 1.00 ± 0.03 .

To examine the contributions of the two polymers in determining phosphate partitioning the individual polymers were exhaustively dialyzed against various concentrations of phosphate at pH 7.5. The phosphate concentrations inside and outside the dialysis bag were then determined (Table I). For the calculation of the salt concentration ratio inside/outside, the inside concentration value was calculated based on the volume of water present, excluding that of the polymer. The volume occupied by the polymer was calculated using a specific volume for dextran, measured pycnometrically and in agreement with Edmond and Ogston (7), of 0.602 ml/g, and for poly(ethylene glycol), 0.837 ml/g (8). The resulting low concentration ratios for PEG 6 show the strong influence of poly(ethylene glycol) on the partitioning of phosphate. Dextran, however, yields an inside/outside ratio of close to one. Increasing PEG concentration produces more one-sided phosphate partitioning which increases in magnitude linearly with polymer concentration (Fig. 6). Within the accuracy of the measurements no clear dependence on PEG molecular weight or on total phosphate concentration was detected. Dialysis of the polymers against sodium chloride results in a lower salt concentration inside the dialysis bag than outside for both dextran and PEG 6. The inside/outside ratios for both polymers differ somewhat, with the PEG 6 value being lower than the dextran value.

DISCUSSION

It is evident from the results presented above that the combinations of mono- and dibasic phosphate salts necessary to provide a physiological pH can have strong, concentration-dependent effects on the properties of dextran/PEG aqueous two phase systems. Increasing phosphate concentration moves the critical points towards lower dextran concentrations, whereas the PEG concentration at the critical point remains nearly unchanged (Fig. 3). The effect of this shift on the tie line length is dramatic near the critical point (Fig. 4), but becomes progressively less obvious in systems with longer tie line lengths, even though phosphate partitions strongly in favor of the dextran-rich phase under these conditions (Fig. 5). Apparently the polymer concentrations are sufficiently high that the PEG-dextran interactions dominate in determining the compositions of the phases (for a TLL of 25% w/w the dextran and PEG monomer concentrations are greater than 5 and 12 times those of the salt, respectively, on a molar basis). The addition of 0.15 M NaCl alone to the phase systems causes only minor changes, a slightly different phase composition yielding a much smaller elongation of the tie lines and a small shift in the critical point. NaCl therefore behaves as an indifferent salt in these systems. This is not because NaCl does not interact with either polymer in solution, however. The data in Table I shows that pure PEG and dextran solutions both effectively reject NaCl to a significant extent, PEG being the more effective of the two. In phase systems, in which the bottom phase dextran concentration is greater than the top phase PEG concentration, these effects apparently are balanced, the result being that no significant preferential partition of NaCl is observed.

Because of its recognized importance in affecting cell partition in these systems, the distribution of phosphate between the phases was of particular

interest. Phosphate partition out of the PEG-rich top phase was observed which increased with increasing tie line length phosphate concentration and ionic strength (Fig. 5). Based on the equilibrium dialysis results, it is to be expected that these results reflect the rejection of phosphate by PEG, with little contribution to the net partition from dextran. This idea is strongly supported by Fig. 6, in which the data from both the equilibrium dialysis and partition experiments are combined. It is seen that, if the difference in PEG concentration between the phases is used as the independent variable, the phosphate partition data from Fig. 5 lies within the scatter of the equilibrium dialysis results. That is, dextran apparently has no effect on the partition of phosphate, in spite of its high concentration in the systems. There is therefore no evidence to support the suggestion (2) that the ionic partitions observed in the phase systems result from the binding of ions to either polymer. Rather, support has been obtained from electrokinetic studies on cells to which dextran and PEG was adsorbed for anion exclusion by the polymers (9). The rejection of ions by PEG has also been observed in NaCl/PEG mixtures by Baldwin et al. (10), albeit at higher temperatures. These workers also observed an increase in the magnitude of the effect with increasing polymer concentration.

The mechanism responsible for the salt rejection by PEG is not known. It is evident from these and other (2) studies that it is the anion which is responsible for the effect, as varying the cationic species produces only small effects. It is possible that water bound to the ether oxygen may be effectively unavailable to participate in the hydration shell of the phosphate. Alternatively, and perhaps more likely, the hydrophobic ethylene group may induce water structures which are less readily able to hydrate phosphate than bulk solvent. It seems clear from the linearity of the polymer concentration dependence of phosphate partition over a wide range of PEG concentrations (Fig. 6), from the

lack of molecular weight dependence and from the activity of even very low molecular weight species in the work of Baldwin et al., that the incompatibility is a local effect involving the interaction of the anion with a relatively small oligomer. Other physicochemical techniques will have to be applied if the mechanism is to be better understood.

The dependence of phosphate partition on the concentration of phosphate and/or NaCl is also of interest (Fig. 5). While the effects are not large, it is clear from the error estimates of the data presented that phosphate partition out of the PEG-rich phase into the bottom phase increases with increasing ionic strength. That the dependence was not detected in the equilibrium dialysis experiments may be due to the relatively narrow range of high phosphate concentrations studied (Fig. 6). The effects of phosphate on TLL, which can be thought of as the reciprocal of the salt partitions observed, did not change much between 0.11 and 0.22 M phosphate (Fig. 4). The anion rejection from the top into the bottom phase can be thought of as a type of Donnan distribution, although the usual situation of ion binding to a macromolecule which is restricted to a particular phase or region of the solution (11) is reversed here. Nonetheless, the ion distribution which results will set up a Donnan potential. Increasing ionic strength suppresses this potential through a screening mechanism, thus allowing more one-sided salt partitions to occur (11). These are the qualitative effects seen at the highest TLL in Fig. 5. Why the 0.15 M NaCl increases the 0.01 M phosphate distribution to a disproportionately large degree is not clear, however.

ACKNOWLEDGEMENTS

This investigation was supported by funds from the Material in Space Program of NASA, Contract No. NAS8-33575, and from USPHS Grant HL 24374 of the National Heart, Lung and Blood Institute.

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FIGURE LEGENDS

Fig. 1: Dependence of the buoyant density of the bottom phase on the dextran concentration of the bottom phase at 22°C in aqueous phase systems of dextran T40 (closed symbols, solid lines or dextran T500; open symbols, dashed lines) with no salt added (o,●), with 0.15 M NaCl (▽,▼), with 0.11 M phosphate, pH 7.5 (□,■) and with 0.22 M phosphate, pH 7.5 (Δ).

Fig. 2: Dependence of the difference in buoyant density on the difference in dextran concentration between the two phases at 22°C. Symbols as in Fig. 1. The solid line represents the linear regression for all values of no salt and NaCl, the dotted line for all values of 0.11 M phosphate and the dotted dashed line for all values of 0.22 M phosphate.

Fig. 3: Phase diagram of aqueous phase systems of dextran T40 and PEG 6 with no salt added (o,●), with 0.15 M NaCl (▽,▼), with 0.11 M phosphate, pH 7.5 (□,■) and with 0.22 M phosphate, pH 7.5 (Δ,▲). Each open symbol represents the critical point which is the intersecting point between the binodial and the line through the midpoints of the tie lines of the same binodial. T = 22°C.

Fig. 4: Change of the TLL with total concentration of sodium phosphate, pH 7.5 in: 7.5% w/w dextran T40, 4.5% w/w PEG 6 (●); 10.7% dextran T40, 4.3% PEG 6 (▲); 14% dextran T40, 5% PEG 6 (■); and the concentration of sodium chloride in 7.5% dextran T40, 4.5% PEG 6 (o).

Fig. 5: Phosphate concentration ratio, top/bottom, at different total phosphate concentrations as a function of the length of the tie line (% w/w) in dextran T40/PEG 6 two phase systems with 10 mM phosphate (o), 0.01 M phosphate and 0.15 M NaCl (●), 0.11 M phosphate (■), and 0.22 M phosphate (▲) total concentrations at 22°C. All systems had a pH of 7.5.

Fig. 6: Phosphate concentration ratio, $\text{phosphate}_t/\text{phosphate}_o$, after dialysis of PEG 6 (o,●) or PEG 20 (Δ,▲) at 22°C against 0.11 M (open symbols) or 0.22 M sodium phosphate pH 7.5 (closed symbols). The line represents the linear regression analysis for all circles and triangles. In addition the partition coefficients of phosphate, $\text{phosphate}_{\text{top}}/\text{phosphate}_{\text{bottom}}$, of the systems in Fig. 5 with 0.11 M (□) and 0.22 M phosphate (■) are plotted versus the difference in the % w/w PEG-concentration, ΔPEG , between top and bottom phase.

Table I: Equilibrium concentration of sodium phosphate, pH 7.5 (PO_4) or sodium chloride (NaCl) inside and outside dialysis sacs.

System	% w/w Polymer	Measured Salt Concentrations (mM)		<u>Inside</u> <u>Outside</u>
		Inside*	Outside*	
I. 0.11 M PO ₄				
Dextran T500	10.65	101.6±1.2	106.9±2.0	1.019
Dextran T500	12.02	104.2±1.2	111.7±1.1	1.011
H ₂ O	—	109.1±0.4	107.4±1.0	1.016
II. 0.22 M PO ₄				
Dextran T500	11.34	199.4±0.3	215.1±0.8	0.995
III. 0.15 M NaCl				
PEG 6	6.69	129.2±0.1	145.5±0.1	0.952
PEG 6	6.25	133.6±0.2	149.1±0.1	0.946
PEG 6	12.02	116.6±0.1	149.1±0.1	0.872
PEG 20	3.50	145.6±1.1	149.0±0.5	1.007
PEG 20	3.92	142.5±1.0	149.0±0.5	0.989
PEG 20	4.70	139.8±0.7	149.0±0.5	0.977
Dextran T500	13.60	134.9±0.3	151.3±1.0	0.976

* \pm Standard error of the mean.

Fig. 1

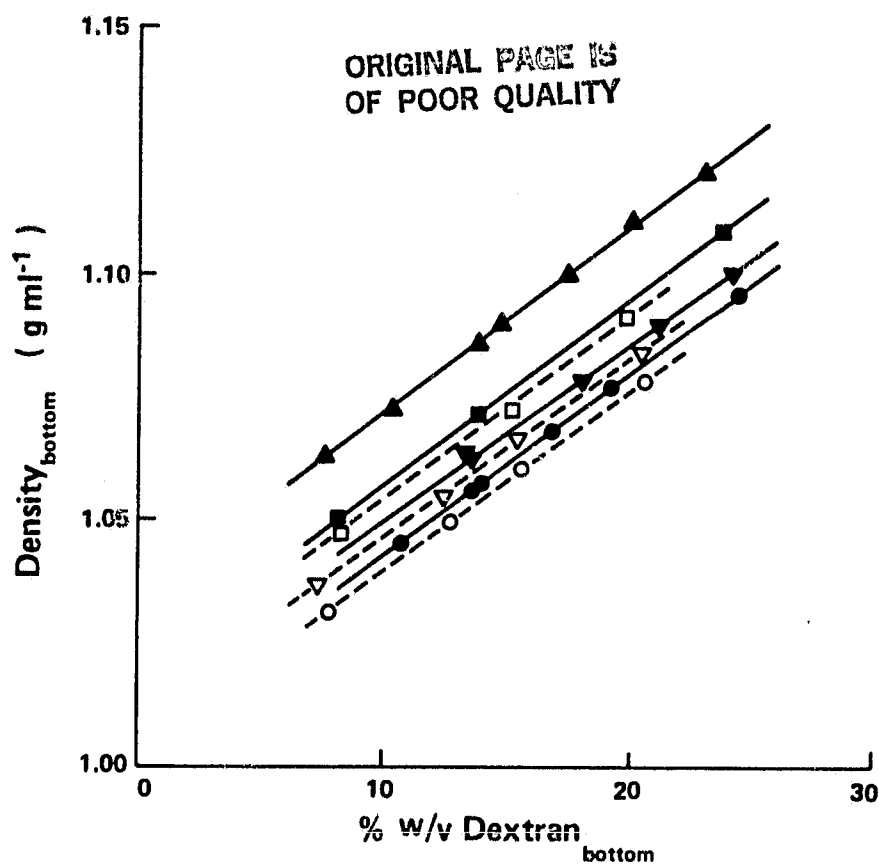
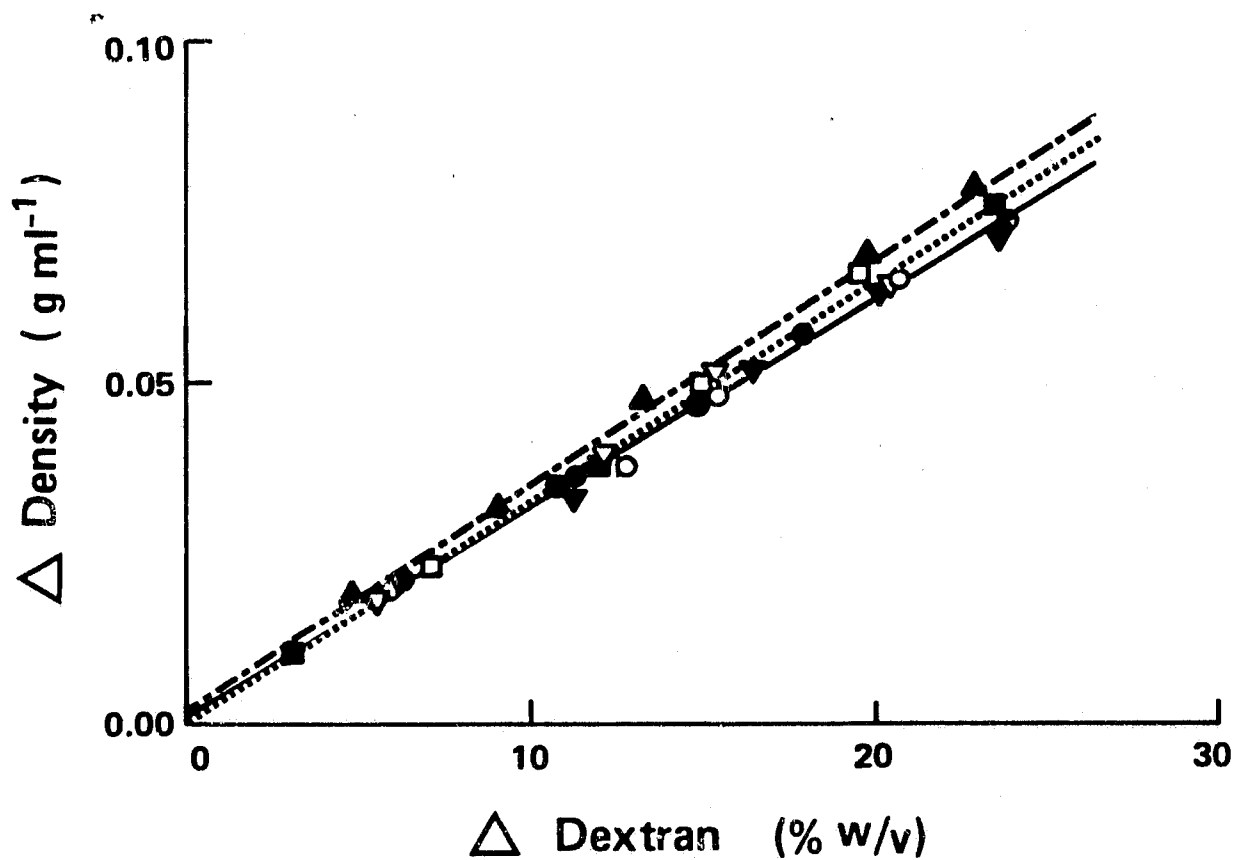


Fig. 2



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Fig. 3

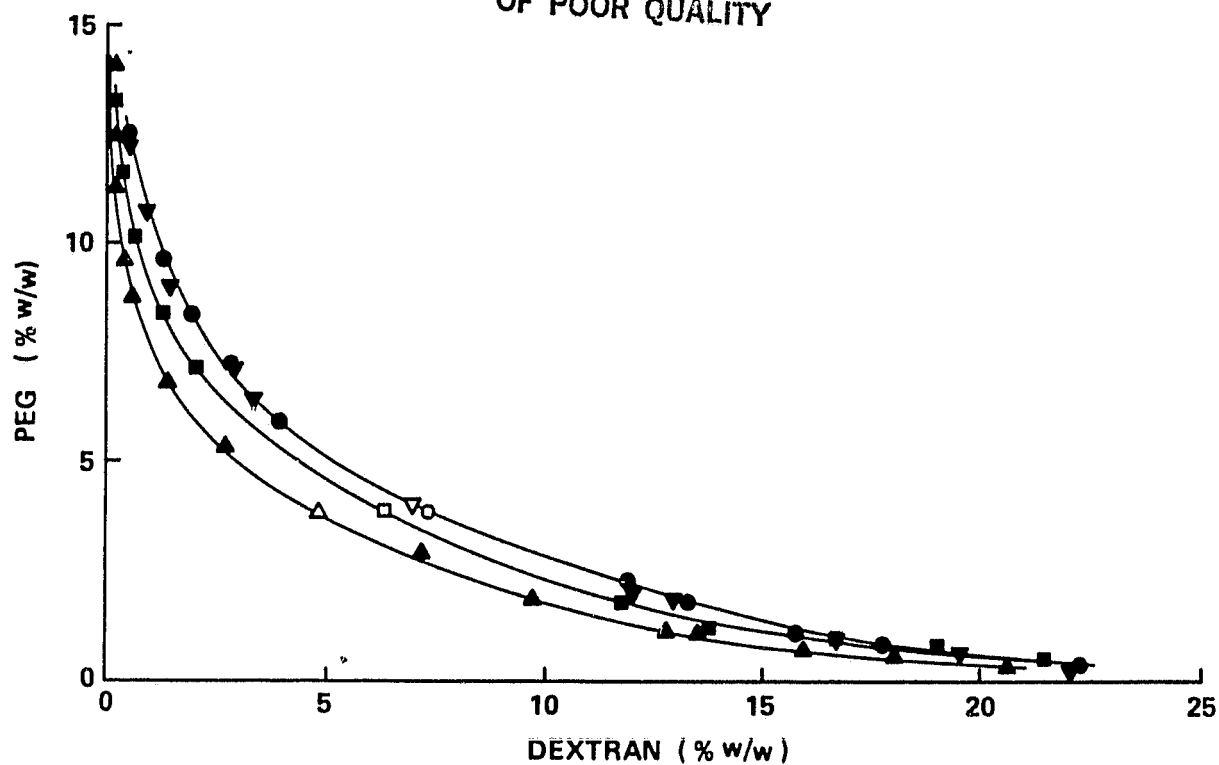


Fig. 4

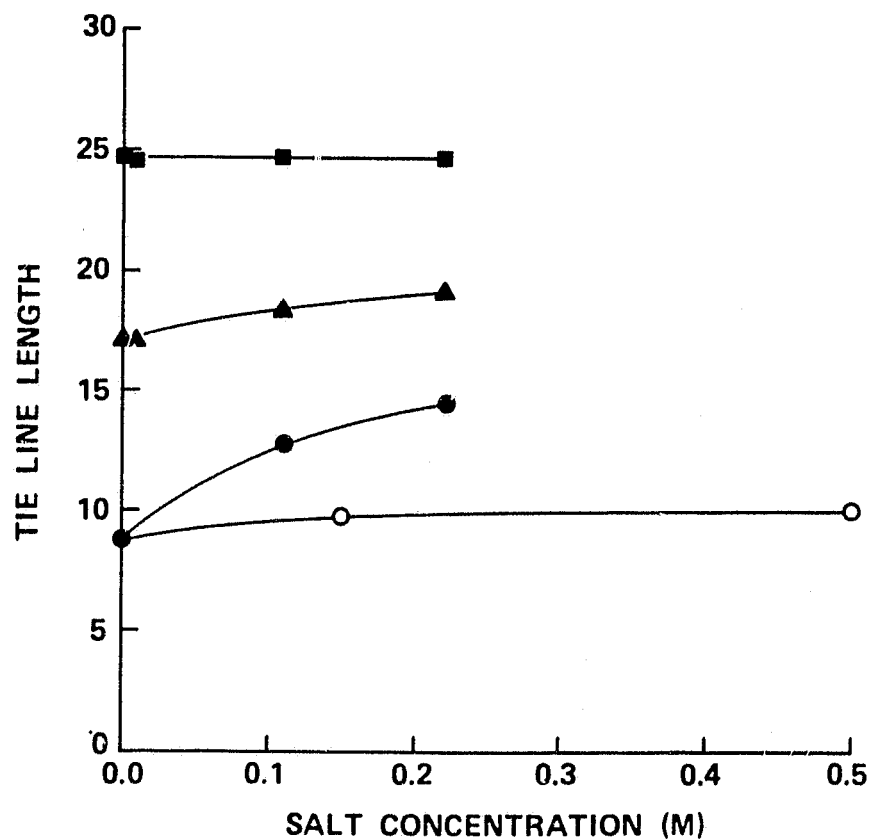


Fig. 5

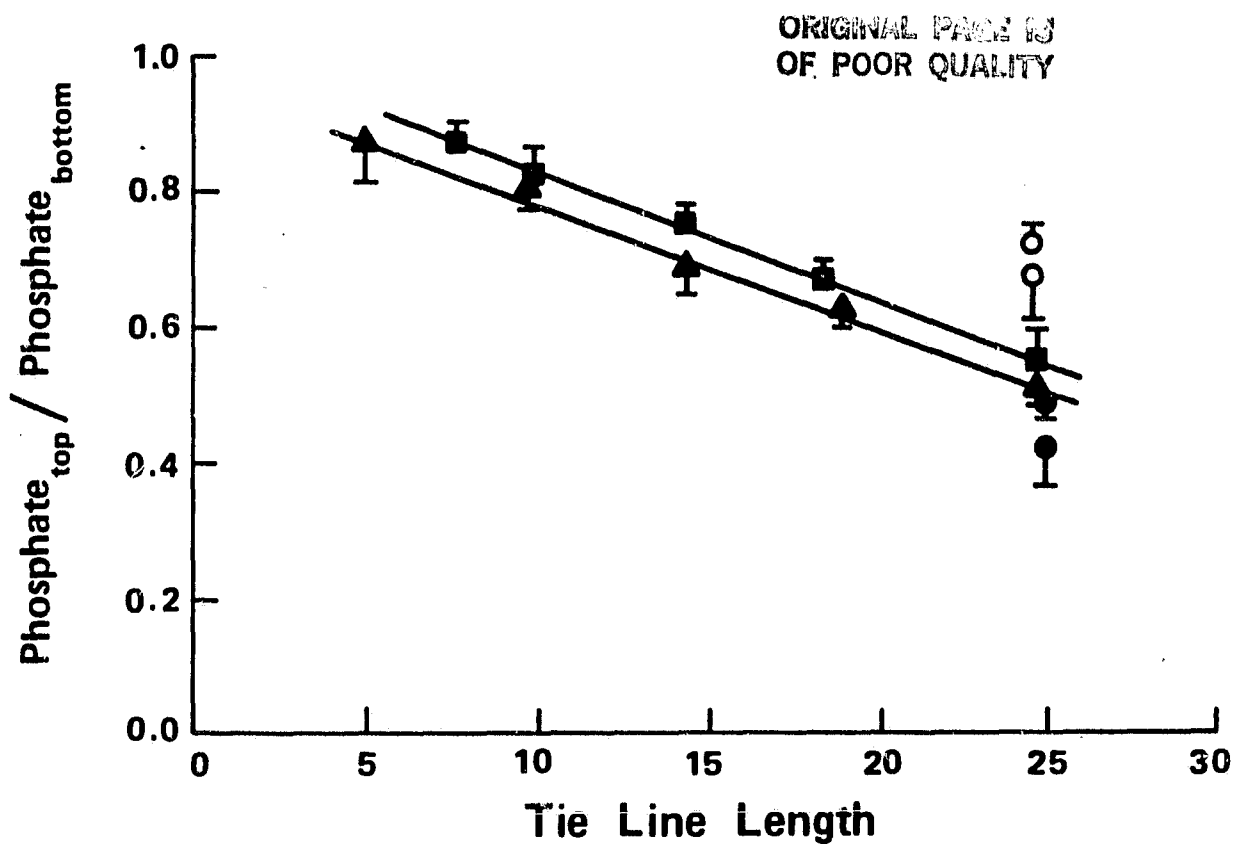
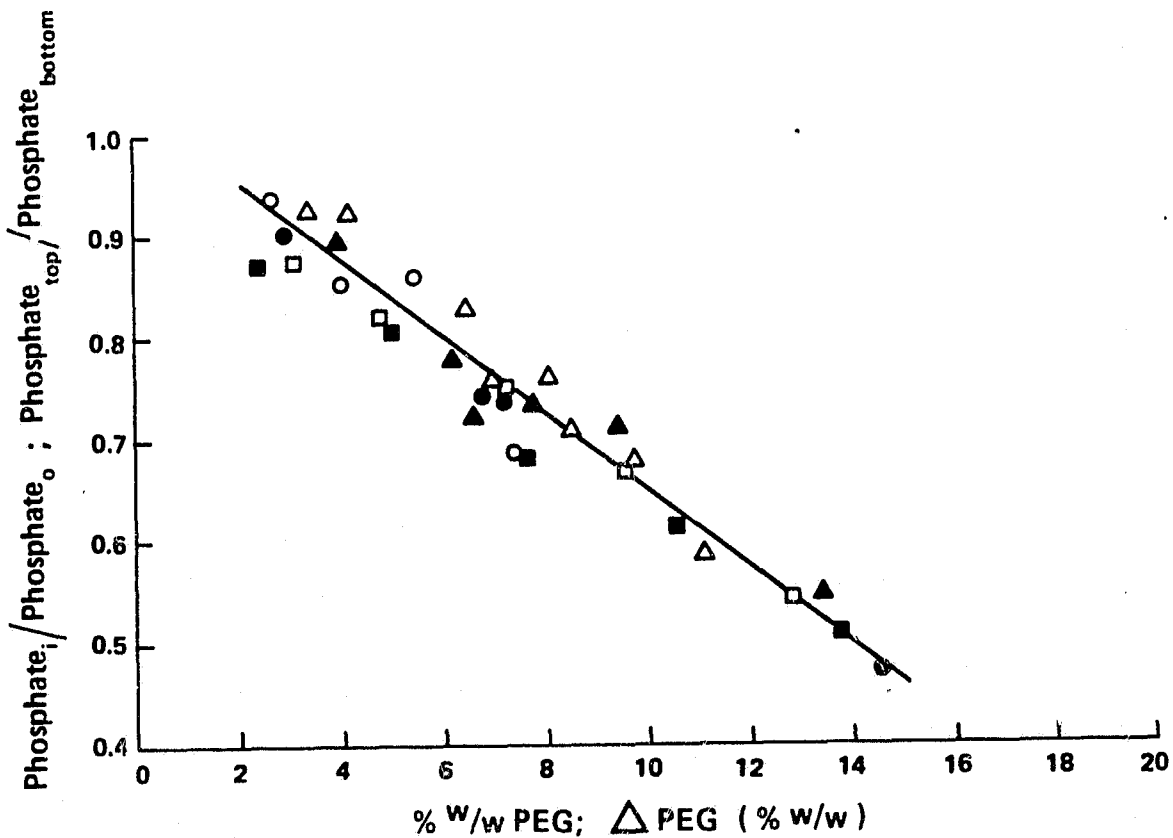


Fig. 6



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STUDIES ON AQUEOUS TWO PHASE POLYMER SYSTEMS USEFUL FOR
PARTITIONING OF BIOLOGICAL MATERIALS

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ABSTRACT

The two phase systems that result when aqueous solutions of dextran and poly(ethylene glycol) (PEG) are mixed above a critical concentration of a few percent provide a useful medium for the separation of biological cell subpopulations via partition between the top, PEG-rich phase and the liquid-liquid phase boundary. Interfacial tensions of such systems have been measured by the rotating drop technique and found to range between 10^{-1} and 10^2 $\mu\text{N m}^{-1}$. The tension was found to depend on the length of the tie line describing the system on a phase diagram, via a power law relationship which differed depending on the concentration of Na phosphate buffer present. The electrokinetic properties of drops of one phase suspended in the other were studied for a variety of systems. It was found that the droplet electrophoretic mobility increased monotonically with phosphate concentration and drop diameter but exhibited the opposite sign from that anticipated from phosphate partition measurements. It was possible to take advantage of these electrokinetic properties and dramatically enhance the speed of phase separation through application of relatively small electric fields.

INTRODUCTION

The two phase systems which form when dextran (poly(α -1,6 glucose)) and poly(ethylene glycol) (PEG) are mixed to form aqueous solutions which are greater than a few percent in each, when appropriately buffered, have proven to be valuable as partition media for macromolecules, sub-cellular organelles and whole biological cells [1-3]. When cells or microscopic particles are introduced to such systems, the systems shaken to emulsify the phases, and allowed to re-settle, it is frequently found that the cells are distributed between the top, PEG-rich phase and the interface between the bulk phases, the dextran-rich phase being empty of cells. The interface therefore acts as a third phase with respect to the distribution of particulates in the systems.

The strength of this procedure as an analytical or preparative process derives from the fact that the partition coefficient (percentage of cells found in the top phase) is determined, ideally, by cell membrane properties which are under direct genetic control and which can vary in ways relevant to cell activity and function. While for macromolecules the partition coefficient is determined purely thermodynamically, for particulates such as cells which do not diffuse to any great degree because of their size (1-50 μm diameter) the determining mechanisms are not well understood.

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Partitioning works well for relatively small cells. An inherent limitation appears for cells which sediment significantly during the time required for the phases to settle, however, since such cells will sediment into the interface, or to the bottom of the tube, before phase separation is complete. By working in a low gravity environment cell sedimentation would be eliminated, but an additional driving force for phase isolation would have to be introduced. We have been investigating the possibility of using an externally applied electric field as a means to collect and isolate the phases. In addition, since the initial stage of the separation process should be partially determined by the interfacial tension of the liquid-liquid interface between the phases, we are studying this parameter as a function of system variables. Our results to date are summarized below.

MATERIALS AND METHODS

Phase systems. These were made up by weight from concentrated stock salt solutions and 25%-35% w/w stock solutions of dextran T40 (Pharmacia Fine Chemicals; lot 7974; $M_w = 41,500$; $M_n = 26,800$) and PEG 6000 (Union Carbide; lot R-529-9104; $M_w = 6000$) in twice glass distilled water. Phase systems were allowed to settle at the desired temperature until neither phase showed visible turbidity, then each phase isolated. Polymer concentrations of the stocks and separated phases were measured polarimetrically (dextran) or refractometrically (PEG) as described [1]. Phosphate concentrations were measured by the stannous chloride method [4] following removal of interfering organics with HNO_3/H_2SO_4 [4]. Phase densities were determined pycnometrically.

Interfacial tension. The rotating drop method [5] was used, employing an apparatus constructed locally, designed after the descriptions of Vonnegut [5], Princen *et al.* [6] and Ryden and Albertsson [7]. The equilibrium lengths of drops of known volume of the PEG-rich phase suspended in the dextran-rich phase were determined with a travelling microscope at measured rotation rates between 300 and 2500 rpm. The interfacial tensions were calculated from the above measurements and the phase densities using the formula and tables given by Princen *et al.* [6].

Phase droplet electrophoretic mobility. Small volumes of one of the isolated phases were injected into large volumes of the other (2-4 μ l per ml), the dispersion mixed gently and introduced into the cylindrical chamber of a microelectrophoresis apparatus (Rank Bros.) equipped with a filar eyepiece micrometer (American Optical Co.). Droplet diameters were measured to $\pm 0.4 \mu$ m using the micrometer, with the field off, before the mobility of each was measured. Mobilities were determined by recording the time necessary for a drop to move a known distance, calculating the velocity, and dividing by the electric field strength applied [8].

Electric field-driven phase separation (FDPS). FDPS was carried out in an apparatus described in detail in the NASA patent documents covering the process [9]. Briefly, the separation cell, made from poly(methyl methacrylate) consists of a sample chamber approximately 0.5 cm W x 5 cm L x 0.2 cm H, its upper and lower boundaries consisting of membranes (Amicon UM2) which separate it from the upper and lower electrode chambers. The design is such that the Rayleigh Number is always less than 100 in the separation chamber under operating conditions, implying that no unstable convection will occur [10]. The electric field is applied vertically via bright Pt wire electrodes. Buffer is continually passed through both electrode chambers to remove the gases generated and to remove heat at the separation

chamber boundaries. A mixed phase system is introduced into the separation chamber via a loading port. The time course of phase separation is followed turbidimetrically through measurement of the intensity of the beam of a ruby laser which traverses the width of the chamber roughly 0.1 cm from the bottom membrane and impinges on a solid state detector. The output from the detector is amplified and displayed on a chart recorder. Introduction of a mixed, turbid phase system scatters most of the beam away from the detector, producing a minimum signal. As phase separation occurs the system clears optically and the transmitted intensity increases correspondingly. It is also possible to isolate the top and bottom halves of the sample chamber since the unit is split in a plane parallel with, and half way between, the upper and lower membranes. The top half can then be slid off the bottom half laterally onto a flat plate, isolating the liquid in the top half of the chamber and leaving the material in the bottom half similarly trapped.

RESULTS AND DISCUSSION

Figure 1 shows the phase diagram for dextran 40, PEG 6000 mixtures at 22°C in the presence of different salts and salt concentrations. It is clear that whereas 0.15M NaCl had no measurable effect on phase separation, Na phosphate buffers (equimolar Na_2HPO_4 and NaH_2PO_4 solutions mixed to produce the desired pH) at pH 7.5 progressively depressed the binodial and critical point as the phosphate concentration was increased. This implies that the polymer concentration differences between two phases formed from systems containing the same total amount of dextran and PEG will be greater the greater the phosphate concentration. A parallel effect has been found in the partition of the phosphate salts in these systems (measured as total P), the phosphate concentration in the dextran-rich bottom phase being greater than that in the top phase and the effect increasing with increasing salt concentration (Table 1). This unequal salt partition produces a Donnan

TABLE 1

Total phosphorous partition in a phase system containing 10.7% w/w dextran 40, 4.3% PEG 6000, pH 7.5

Phosphate conc'n (M)	Tie line length (% w/w)	Phosphate partition coefficient ^a
0.22	19.0	0.622
0.11	18.4	0.665
0.01	16.3	0.754

^a) Phosphate partition coefficient = molar concentration ratio, top/bottom

potential between the phases [11] which is believed to influence the partition behavior of charged particles and cells.

The interfacial tensions measured at 22°C for the systems analyzed in Figure 1 are given in Figure 2. The parameter which characterizes the phase system in this plot is the tie line length (TLL), the distance on the phase diagram between the two points on the binodial which give the composition of the separated phases. It is seen that the tensions are in the range 10^{-1}

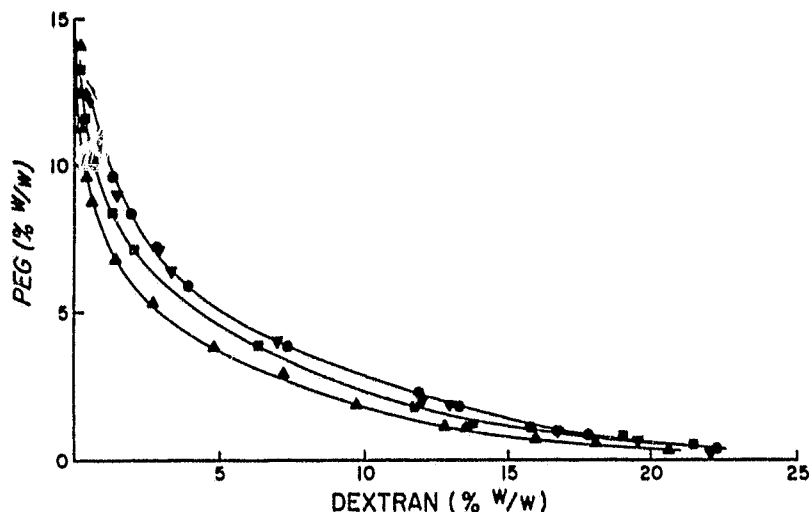


Fig. 1. The dextran T40, PEG 6000 phase diagram at 22°C. Systems contained: (●) no salt; (▼) 0.15 M NaCl; (■) 0.11 M Na phosphate buffer, pH 7.5; (▲) 0.22 M Na phosphate buffer, pH 7.5.

to $10^2 \mu\text{N m}^{-1}$ (10^{-4} - 10^{-1} dyne cm^{-1}). The log-log plots are strictly linear over the range of compositions examined, the slopes being 3.64 (no salt), 3.77 (0.11 M phosphate) and 3.52 (0.22 M phosphate). Hence the tension varies as TLL raised to these powers.

The conclusion that the interfacial tension shows a power law dependence on TLL is at variance with that of Ryden and Albertsson [7] who found, working over a more limited composition range, that the log of the tension depended directly on TLL. Such a relationship clearly could not apply at TLL = 0, however. In fact, Ryden and Albertsson's data agrees quite well with ours where the two sets overlap.

The other noteworthy feature of Figure 2 is that the systems containing phosphates exhibit unique behavior for each concentration. The effect of phosphate is not just to drive the system further from the critical point and increase the TLL. Systems with the same tie line length have higher tensions the higher the phosphate concentration. This suggests that the value of the interfacial tension reflects to some extent the magnitude of the salt concentration gradient across the phase boundary as well as those of the two polymers in the systems.

The results of electrokinetic studies on droplets of one phase suspended in the other for various systems are given in Figures 3 to 5. Figure 3 shows that drops of the top, PEG-rich phase exhibited a negative electro-phoretic mobility and drops of the bottom, dextran-rich phase a positive in experiments in which one or the other was the disperse phase. The magnitude of the mobility increased with increasing phosphate concentration (in the presence of sufficient supporting NaCl to maintain the ionic strength approximately constant). Remarkably, the sign of the mobility is opposite to that expected from the phosphate partition and the Donnan potential,

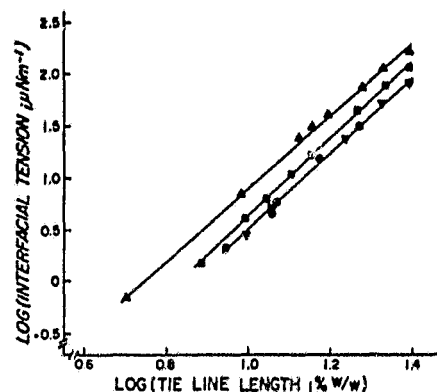


Fig. 2. Log-log plot of interfacial tension as a function of tie line length; $T = 22^{\circ}\text{C}$; symbols as for Fig. 1.

dextran-rich drops exhibiting a positive mobility in spite of containing a higher phosphate concentration than the surrounding PEG-rich phase.

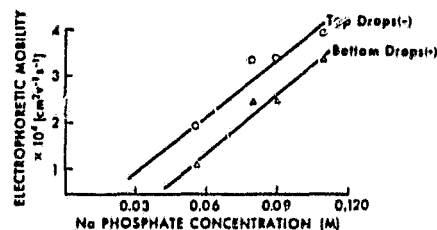


Fig. 3. Electrophoretic mobility of droplets as a function of Na phosphate buffer concentration, pH 7.5, in a phase system containing 7.5% dextran T40, 4.5% PEG 6000 plus, for points from low to high phosphate respectively, 0.05 M, 0.04 M, 0.03 M and 0 NaCl; drop diameter 8-11 μm ; $T = 25.0^{\circ}\text{C}$. Top drops have a negative mobility, bottom drops a positive mobility.

A second interesting feature of the droplet electrokinetic behavior is shown in Figures 4 and 5 where the dependence on drop size is illustrated. The mobility is a monotonically increasing function of drop diameter in all cases and appears to increase linearly with diameter over the size range accessible. In all but one case (bottom phase drops in Figure 4) linear extrapolation of the data predicts a finite mobility as the diameter approaches zero. These results may imply solid-like behavior as the drop size diminishes and surface tension effects become dominant, since solid particles would not exhibit size-dependent mobilities under these conditions [12]. Alternately, the dependence of drop mobility on diameter may be nonlinear and the mobilities may approach zero below the sizes studied in this work. In the exceptional case a change in sign in mobility is predicted at about 4 μm diameter. No actual reversal was observed, however, and the source of the effect is obscure at present.

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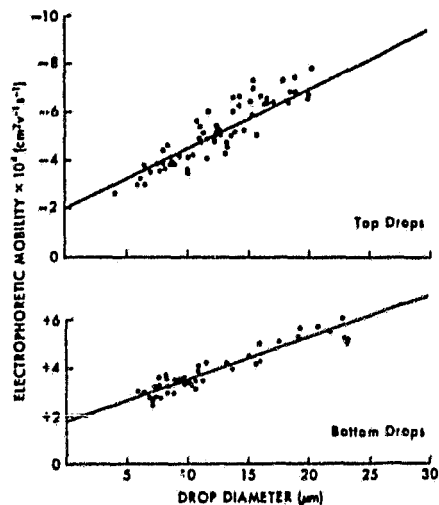


Fig. 4. Drop mobility vs diameter for the system containing 7.5% dextran T40, 4.5% PEG 6000 and 0.11 M Na phosphate, pH 7.5; $T = 25.0^\circ\text{C}$.

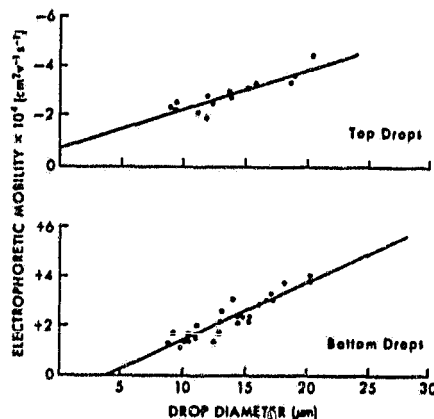


Fig. 5. Drop mobility vs diameter for the system containing 9% dextran T40, 6% PEG 6000 and 0.11 M Na phosphate, pH 7.5; $T = 25.0^\circ\text{C}$.

The studies illustrated, combined with the results of work in progress, make it clear that there is no simple relationship between the electrokinetic behavior and the distance of the system composition from the critical point. Probably two competing effects contribute to this behavior, mobility reduction due to viscosity increases for systems ~~away~~ from the critical point, and mobility increases associated with more ~~extreme~~ phosphate partition in systems with longer tie line lengths (Table 1). In spite of the unanticipated sign of the mobility, its magnitude appears to correlate with the value of the phosphate partition coefficient. In the systems examined to date, viscosity effects eventually take over as progressively more concentrated systems are used, however, and the mobilities are reduced (Figure 5).

The dependence of drop mobility on size is important with respect to the use of electric fields to produce phase localization in these systems. As an emulsified phase system begins to separate, droplet coagulation occurs. Since the settling velocity of isolated drops increases with the square of the radius [9], this leads to reasonably rapid separation. That the electrophoretic mobility of phase drops also increases - albeit more weakly - with increasing size suggests that qualitatively similar effects on phase separation might result from the application of an electric field. In fact, because of the magnitudes of the coefficients involved, with an applied field of 4 v cm^{-1} the electrophoretic velocity of, for instance, the bottom phase drops described in Figure 4 would be greater than the settling velocity for drops of up to 1.7 mm diameter.

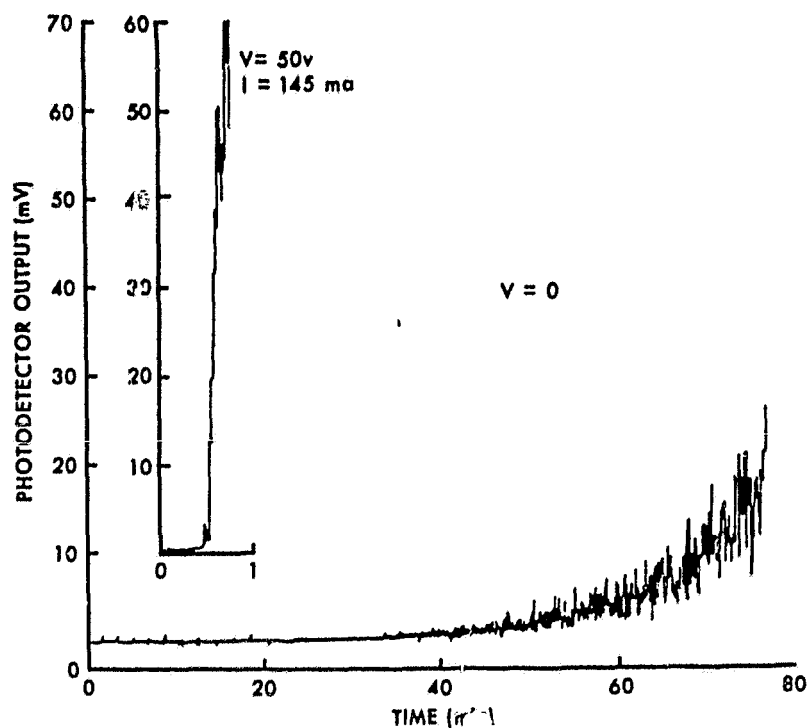


Fig. 6. Optical clearing of a 7.5% dextran T40, 4.5% PEG 6000, 0.11 M Na phosphate phase system in the absence (lower trace) and presence (inset) of an electric field of 4.5 v cm^{-1} , top electrode positive. Note expanded time scale in inset.

Figure 6 illustrates the results of an experiment which demonstrates the feasibility of FDPS. The lower trace describes the optical clearing of a phase system containing 10% v/v bottom phase, 90% top, in the absence of an applied field. The inset shows the clearing observed when a field of about 4.5 v cm^{-1} is applied, top electrode positive. The turbidity decreases between one and two orders of magnitude more rapidly under these conditions, suggesting that droplet electrophoresis has indeed resulted in a very rapid separation of the phases.

This interpretation is fully supported by measurements of the composition of solutions isolated from the top and bottom halves of the chamber following field application for various lengths of time. These measurements demonstrate that at a 1:1 top to bottom phase volume ratio the phases are effectively separated in less than one minute at field strengths 4 v cm^{-1} . They also show that, with the optical path length and geometry

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used, optical clearing occurs at a considerably later stage in the process than does the separation of most of the volume of each phase. Apparently the optical properties of the systems are dominated at later times by the presence of dispersions of very small drops of one phase in the other that do not represent a significant fraction of the total phase volume. Bulk phase separation occurs in our FDPS chamber with only an increase of a few percent in light transmission, therefore.

The above results demonstrate that dextran-PEG phase systems containing significant concentrations of phosphate buffer possess a variety of interesting equilibrium and electrokinetic properties. The physicochemical bases of the salt partition, and particularly the electrokinetic behavior of the phase systems, are not yet understood, although a theory describing phase drop electrophoresis is currently under development (see contribution of S. Levine in this collection). The electrokinetic properties are such that application of electric fields can enhance the rate of phase separation many fold. The prospects for applying FDPS to cell separation problems therefore appear to be good.

ACKNOWLEDGMENTS

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